THE DEVELOPMENT OF THE POLLEN OF SARRACENIA

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(WITH PLATE V)

During March 1907, through the courtesy of Dr. J. M. Macfarlane, I had the opportunity of gathering material for this study from the fine collection of sarracenias in the greenhouses of the University of Pennsylvania. The species examined were *S. flava*, *S. purpurea*, *S. variolaris*, *S. rubra*, and *S. psittacina*, besides the hybrids *S. flava* × *variolaris* and *S. flava* × *purpurea*. A close similarity prevails in the pollen of the different species, nor does any marked difference appear in the hybrids. Abnormalities of division among the latter are practically absent.

The material consisted of flower buds fixed in chrom-acetic acid. Thin sections of the anthers were stained by the iron-hematoxylin method and with safranin and malachite green.

The nucleus of the pollen mother cells contains an intensely staining nucleolus surrounded by a clear space. With safranin and malachite green the color of the nucleolus is red. The surrounding plasm (linin) does not stain deeply, and shows a trace of fibrillar structure (*fig. 1*). The approach of the synapsis stage is indicated by a marked and apparently rather sudden growth in size of the nucleus. The nucleolus also becomes larger, and the clear space surrounding it less well defined but wider. The linin shows more definite indications of a network (*fig. 2*). A few deeply staining granules now appear within the linin; these increase in number as the network develops (*fig. 3*). Occasionally granules can be seen in close proximity to the nucleolus (*fig. 3*), which become larger and irregular in shape, and show a tendency to accumulate in the vicinity of the nucleolus and to surround it (*figs. 4–7*). In such nuclei there is sometimes evident a similarity in size of pairs of chromatin clumps. Thus in *fig. 4* there are visible two masses of chromatin, one on each side of the nucleolus of about the same size; while farther removed from the nucleolus there are two others, smaller than the first, but approximating each other in size. Similar relations are seen in *fig. 5*.

The chromatin next becomes densely massed around the nucleolus, and the irregular clumps elongate more or less into rods. So compactly do they come to lie around the nucleolus that it is completely hidden from view, and only at the margin of the somewhat rounded mass can a threadlike appearance of the chromatin be observed. The chromatin is now gathered together at one side of the nuclear space and the synapsis is well established (*figs. 7, 8*). The nucleolus gradually emerges from the center and takes a position to one side of the chromatin. When it has completely emerged its staining capacity is seen to have diminished (*figs. 9–12*). In some cases a bipartite arrangement of the chromatin is observable (*figs. 11, 12*).

The synaptic mass in the cells at one end of the anther sac now begins to lose its power to take the stain uniformly, and an appearance of dark clumps lying in a paler matrix is produced. The nucleolus is also usually vacuolated (*figs. 13, 15, 16*). The dark bodies approach the nucleolus and it seems to sink into or to be surrounded by the paler area. Within its substance is frequently seen material staining deeply like the bodies without; often this forms a partial ring around a vacuole (*fig. 16*). The nucleolus now becomes well filled with darkly staining material, while the substance without, which takes a similar stain, decreases in amount; it finally disappears entirely. At the same time the nucleolus has become entirely black with safranin red, and lies wholly within the paler substance (*figs. 17–19*). This condition was observed in *S. purpurea* and in *S. flava*×*purpurea*. The other species did not present suitable material for this stage.

The linin next begins to extend toward the circumference of the nucleus and to be converted gradually into a network (*figs. 20–22*). Dark granules, purple with safranin and malachite green, now appear in the network, and at the same time the nucleolus again shows vacuoles (*figs. 23, 24*). It seems to go through in a reverse order its former series of changes, dark material within the nulceolus often surrounding a central vacuole (*fig. 23*). Sometimes the nucleolus presents the appearance shown in *figs. 23a* and *23b*. At one focus three darkly staining spheres can be seen, whereas at a slightly higher level these spheres are replaced by vacuoles. In one case a body closely resembling the nucleolus was observed lying just outside the

nucleus, as if it had been accidentally displaced in cutting the section (*fig. 28*). If this be true, it would seem to indicate that the nucleolus is more or less independent in structure and has a resistant wall of its own. Occasionally, especially in *S. purpurea*, two nucleoli are found, one slightly smaller than the other (*fig. 24*). Sometimes (observed in *S. flava* and *S. variolaris*) the central portion of the nucleolus appears dark, the peripheral portion lighter, while from the central mass are budded off minute spheres (*figs. 22, 25*); or half the nucleolus may stain deeply, the other half faintly (*fig. 27*). The chromatic network eventually consists of fine granules distributed on delicate linin fibrils (*fig. 27*). At this stage the nucleolus, in preparations stained with safranin and malachite green, colors purple, inclosing darker purple granules, the vacuoles pinkish. The nucleoli of the tapetal cells, on the other hand, stain red.

The conversion of the network into chromosomes during the prophases of the first maturation division is illustrated in *figs. 29–31*. A longitudinal splitting of the chromatin granules and linin fibers is clearly seen, the halves thus formed often showing a tendency to spread apart (*fig. 30*). The chromosomes occur in pairs, which come to lie parallel to each other (*figs. 29–31*). They gradually shorten and thicken, so that finally little difference is evident between the length and breadth. That the spindle is formed, at least in part, from the linin fibers would appear from *fig. 32*, which represents a very late prophase. The spindle fibers when fully formed are sharply defined and show minute swellings. Two fibers may be seen attached to a chromosome in *fig. 33*. The separation of the daughter chromosomes is represented at its inception in *fig. 34*. The U-shaped chromosome would seem to correspond to the more elongated body of similar shape in *fig. 29*, and if this is true the first division separates univalent chromosomes and is reducing. The nucleolus persists at its full size until a late prophase. The total number of chromosomes in *S. variolaris* and in *S. rubra* is apparently twelve. The material did not present stages suitable for determining the number in the other species studied. SHREVE (**10**) reports twelve in *S. purpurea*.

After the first division of the chromatin, the daughter nuclei undergo no reconstruction, but arrange themselves for the succeeding mitosis (*figs. 36, 37*). The chromosomes seem to become somewhat

elongated and still manifest a longitudinal split. The second division separates the chromosomes along the longitudinal split and is therefore equational (*fig. 37*). The daughter nuclei are now reconstructed. A large dark nucleolus appears in each, while the rest of the nucleus is occupied by a linin network and scattered chromatin granules. *Fig. 38a* would seem to indicate that these granules are formed by the breaking-up of larger clumps. The nucleolus stains red, the network purplish with safranin and malachite green. During mitosis the chromosomes are bright red, but after reconstruction this color is gradually lost. The cytoplasm, which has remained undivided, now constricts and the four cells of the tetrad are formed (*fig. 40*).

DISCUSSION

THE NUCLEOLUS.—This term, in its limited sense, has been applied to bodies within the nucleus more or less spherical in shape and staining unlike the chromatin (WILSON **12**). The great diversity in the observations on the behavior of this body has given rise to several theories concerning its origin and function. MONTGOMERY (**6**), in his comprehensive review of the literature on this subject in both plant and animal cells, and WAGER (**11**), in his discussion of the nucleolus in plant cells, mention a large number of authors whose observations lead them to believe that there is a connection between the nucleolus and the formation of chromatin. This view is supported, not only by the morphological relations of nucleolus and chromatin, but also by researches on their chemical relations. Another opinion is that the nucleolus has something to do with spindle formation. STRASBURGER (**9**) and NĚMEC (**7**) adopt this view. Many writers on the Protista also report kinoplasmic material arising from the nucleolus. A third theory maintains that the nucleolus contains material which is periodically expelled into the cytoplasm. By some, notably HÄCKER (**3**), this is considered an excretory function. Others believe that the phenomenon is correlated with the appearance in the cytoplasm of substances having there a definite physiological duty.

My own observations afford support to the first of the explanations outlined above. The growth in size of the nucleolus simultaneously with the appearance of darkly staining granules in the nucleus, the accumulation of this material in the nucleolus, and its expulsion,

lead irresistibly to the conclusion that the formation of the chromatin is intimately connected with nucleolar activity. The relations of the nucleolus and the chromatin in the prophases of the first maturation division again plainly suggest an elaboration of chromatin by the nucleolus. The fact that at this time the nucleolus stains purple, whereas ordinarily it stains red with the safranin and malachite-green combination, indicates that a chemical change of some sort is taking place within it. The globules of material thus elaborated escape into the nuclear sap, there to be absorbed by the linin and distributed along its threads. GREGORY (2) was led to a somewhat similar view by his study of the pollen development in sweet peas. His observations indicate that "the nucleolus receives and stores the bulk of the chromatin during the resting periods which intervene between successive mitoses."

The varying observations concerning the behavior of the nucleolus give rise to the opinion that it has not the same definiteness of function as the chromosomes and centrosomes. Since there is certainly an exchange of material between cytoplasm and nucleus, and within the nucleus progressive and regressive changes in chemical constitution of its contents, it would seem that a rounded body staining unlike chromatin and therefore designated nucleolus might frequently arise, but representing at different times and in different cells a different physiological activity. The precise nature of the metabolism of the cell, it is natural to suppose, varies according to the kind of cell and the species of plant or animal, and therefore the nucleoli may be expected to vary in appearance and in their relation to the chromosomes or other constituents of the cell. That this may be the case in the ova of related species of animals has been demonstrated by MCGILL (4) in her studies of the dragon flies. In some forms apparently the nucleolus elaborates material for the use of the chromatin and thus granules and irregular masses occur within it. In others perhaps the material is not greatly transformed in the nucleolus, but passes out into the nuclear sap there to be absorbed and utilized by the chromatin. In the latter instance the connection between the nucleolus and the formation of chromatin would be difficult of demonstration. Thus such evidence as is given by DUBLIN (1) of the non-participation of the nucleolus in chromatin formation is negative

in character, and does not necessarily disprove this function of the nucleolus.

SYNAPSIS AND THE INDIVIDUALITY OF THE CHROMOSOMES.—The chromatin in this stage is so densely massed together as to make it impossible to distinguish individual chromosomes and to determine whether or not there takes place a conjugation of chromosomes as required by MONTGOMERY'S hypothesis. According to the researches of MIYAKE (**5**) and OVERTON (**8**), supporting STRASBURGER'S observations, the chromosomes are preceded in the pollen mother cells by prochromosomes, centers around which the chromatin collects, and which correspond to the chromosomes in number. In Sarracenia I was unable to satisfy myself that the points around which the chromatin collects were constant in number or that the number was the same as that of the chromosomes.

The behavior of the nucleolus just described is not entirely favorable to the idea of the individuality of the chromosomes as such. If any morphological differentiation of the nucleus into areas corresponding to the chromosomes really exists at this time, its basis might possibly lie in the linin. The chromatin and nucleolus at the stage represented in *fig. 19* stain red with safranin, while the linin takes the green. This stain serves to bring out a fibrillar structure in the linin. It is well known that the chromosomes differ greatly in staining capacity at different periods in their development. At certain stages they seem to lose a part of their substance and to diminish in size as well as in staining capacity; whereas at another period they are able to absorb material, grow in size, and color more deeply. The phenomena described might therefore be explained on the assumption that the morphological basis of the chromosomes remains in the linin, while that part of their substance which causes them to color deeply is absorbed by the nucleolus.

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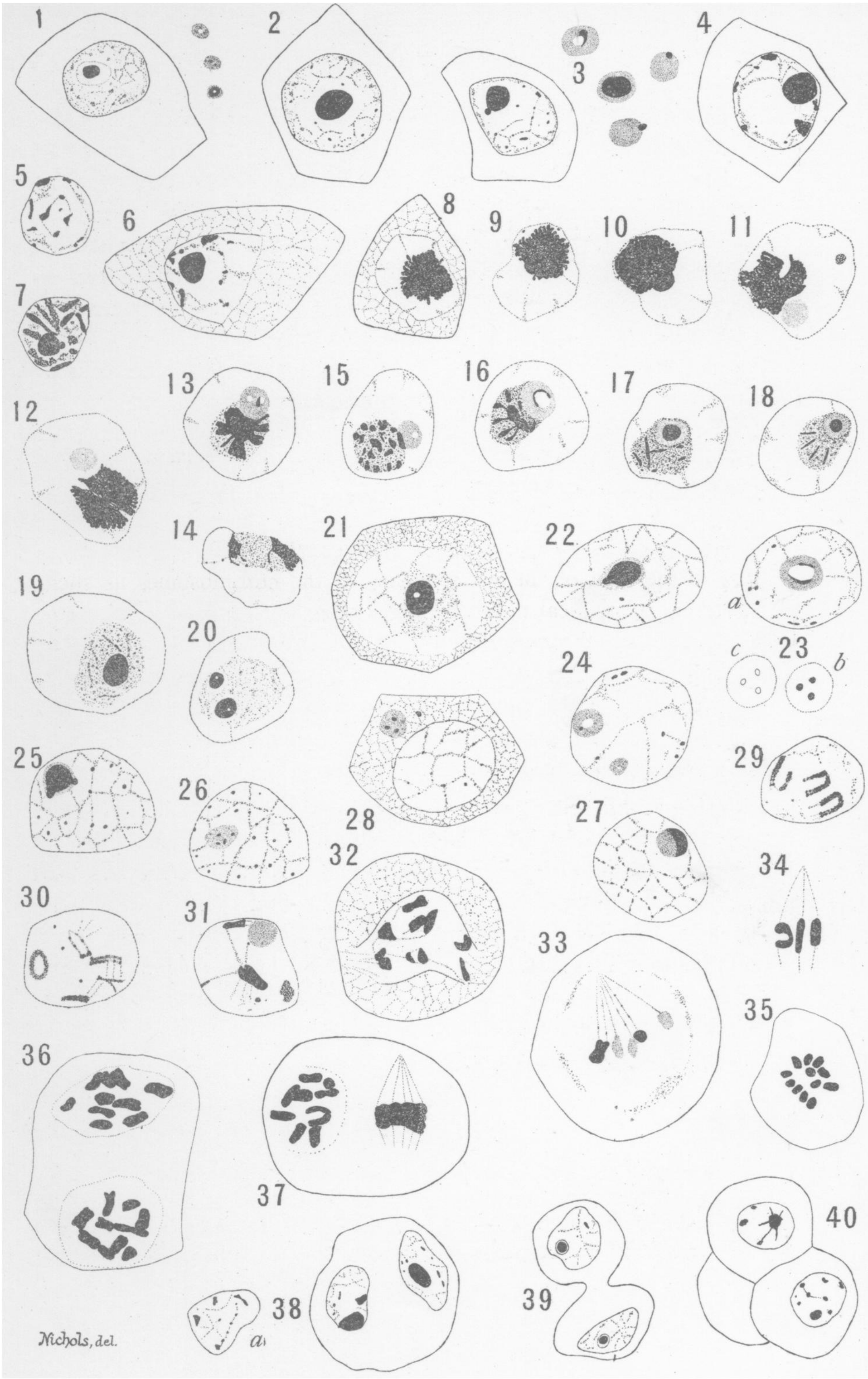

NICHOLS on POLLEN of SARRACENIA

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EXPLANATION OF PLATE V

The drawings were made with the camera lucida, ocular 6, oil immersion objective $\frac{1}{12}$, enlarged to twice the original size and reduced one-half.

Figs. 1–10, S. purpurea; 11, 12, 27, and *34, S. flava; 13–21, 23, 24, 26, 28, S. flava×S. purpurea; 22, 25, 29–32, 33, 35–40, S. variolaris*

FIG. 1. Resting pollen mother cell; *a, b, c,* nucleoli.

FIGS. 2–8. Transition to synapsis; *3a, b, c, d,* nucleoli; *fig. 5,* pole view; *fig. 6,* side view of approximately the same stage.

FIGS. 9–12. Synapsis; gradual emergence of the nucleolus.

FIGS. 13–19. Absorption of chromatin by the nucleolus; *fig. 14,* section below the level of the nucleolus.

FIG. 20. Extension of the achromatic substance.

FIGS. 21–28. Formation of the chromatic network; *figs. 23b* and *c,* nucleoli.

FIGS. 29–32. Prophases of the first maturation division.

FIGS. 33, 34. First maturation spindles.

FIG. 35. Pole view of the same.

FIGS. 36, 37. Second maturation division.

FIGS. 38–40. Completion of the cell divisions and formation of cell walls.